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Stable isotope analysis in archaeological studies has greatly added to our knowledge of past populations. Based upon the premise of 'we are what we eat and drink', chemical analysis of archaeological bone and tooth samples has allowed researchers to reconstruct palaeodiets and individual place of origin (Ambrose 1993; Jim *et al.* 2004). This information may lead researchers to discover other aspects of culture such as social, political, and economic organization, as well as aspects of biological status such as demography, health and physiology.

The potential for archaeological research with stable isotopes was first recognized in the 1960s when Robert Hall noted that maize and other grasses produce unusually young radiocarbon dates (Ambrose 1993). Since that time, stable isotope analysis has become a fairly routine addition to archaeological research with new technological improvements ever increasing the accuracy and variety of information gained. One such technological advancement is the use of laser ablation for carbon and oxygen analysis of hydroxyapatite from bone and teeth. Although not yet in common use, this technique has a number of advantages over traditional methods that require more sample volume and preparation time. As such, there are a number of potential applications in stable isotope analysis of archaeological material (Cerling and Sharp 1996; Jones *et al.* 1999). This paper will give a brief history of isotope analysis using traditional methods and to outline the advantages of using the new technique of laser ablation in conjunction with conventional mass spectrometry.

Principles of Isotope Analysis

An isotope is a form of an element that has the same number of electrons and protons as the most common form but differs in the number of neutrons. The chemical properties of isotopes are essentially the same but this difference in neutrons creates a mass difference. This leads to what are termed 'heavy' and 'light' isotopes. Light isotopes tend to enter into chemical reactions faster than heavier ones, leading to a discrimination or *fractionation* against one

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isotope. Biological tissue will contain more of one isotope than the other (Jim *et al.* 2004). The difference in the abundance of heavy to light isotopes is what is measured during isotope analysis. As the differences are so small, by convention all isotope data are reported as ratios using delta notation with units of per mil (‰). For example, delta (δ) ^{13}C refers to the ratio between ^{13}C and ^{12}C while $\delta^{18}O$ indicates the ratio between ^{18}O and ^{16}O (Faure 1986; Ambrose 1993).

As indicated earlier, the use of stable isotopes in palaeodietary reconstructions is built upon the notion that the isotopic composition of animal tissue is a direct reflection of the isotopic composition of the food and water ingested by the individual (Ambrose 1993; Jim *et a*l. 2004). The main elements used for dietary analysis are carbon (C) and nitrogen (N). Analysis of bone and teeth maybe be used to indicate the type of food being consumed, i.e. C_3 (crops such as wheat, barley, rice, fruits and vegetables) versus C_4 (tropical grasses, millet, maize) plants; the amount of meat in the diet; and the source of meat protein; i.e. terrestrial versus marine (Ambrose 1993; Bell *et al.* 2001).

Oxygen (O) is used for palaeoclimate reconstructions and migration studies as it is also used to determine the place of origin of individuals. The O isotope values in hydroxyapatite are in equilibrium with body water which is a function of the isotope values of the water imbibed by the individuals and which is in turn a function of precipitation isotope values (Ambrose 1993; White and Spence 1998; Jones *et al.* 1999). Individuals reflect the oxygen isotopic composition of the environment in which they were born. In one study, archaeologists were able to identify the geographical origins of individuals from a particular locale within the site of Teotihuacan using oxygen isotope analysis and comparative populations (White and Spence 1998).

Traditional methods of isotope analysis of archaeological material have been in use since it was first noted that stable carbon isotopes could be used for dietary studies (Sullivan and Krueger 1981). Isotopic analysis may be performed on essentially any tissue created through metabolic processes, including breath, blood, hair, skin, and fingernails. As most of this fragile material preserves poorly and is rarely recovered from archaeological sites, bone and teeth are most often the focus of study for researchers. Both the organic collagen and inorganic apatite may be analyzed for use in dietary reconstruction as both contain carbon and collagen contains nitrogen. However, only the mineral portion, the hydroxyapatite contains both carbon and oxygen. Although it may be used in dietary studies in conjunction with collagen, it alone is used for palaeoclimate and migration studies (Ambrose 1993; Fricke and O'Neil 1996; Jones *et al.* 1999). The basic chemical formula of hydroxyapatite is: $Ca_{10}(PO_4)_6(OH)_2$, with two potential modifications: structural carbonate (HCO_3) is generally substituted for phosphate (PO_4) in the crystal structure at about 4 – 5% by weight and carbonate (HCO_3) from the surrounding burial environment may adhere onto the crystal surfaces (Lee-Thorp 2002). This is significant as both the carbonate and phosphate in the apatite may be analyzed for oxygen isotopes (Cerling and Sharp 1996).

As indicated, hydroxyapatite from archaeological material is used for both palaeodietary reconstructions and palaeoclimatic and migration studies. It may be found in both bone (~ 70%) and teeth, although at a much higher percentage in enamel (~ 98%) (Ambrose 1993). Generally, enamel is the preferred material for analysis as it tends to preserve better than bone and is recovered more often from archaeological sites. As well, it is suspected to suffer less from potential post-depositional chemical alteration due to its lower organic composition and higher degree of crystallinity (Lee-Thorp and Sponheimer 2003). Traditional methods of hydroxyapatite oxygen extraction and analysis are typically lengthy and somewhat complicated. Generally, 10 – 40 mg of sample is treated with acid to dissolve the bone; phosphate is reprecipitated with silver phosphate (Ag_3PO_4), and then reduced at high temperatures with a fluorinating compound such as BrF_5 to produce CO_2 or O_2 gas. This gas is then analyzed using a mass spectrometer which determines the isotope ratios (Faure 1986; Ambrose 1993; Jones *et al.* 1999).

However, this method requires large amounts of sample and is both destructive and time consuming. Recently devised methods of laser ablation have enhanced both the spatial resolution and rapidity of isotope analysis allowing for more detailed studies and use of rare or potentially isotopically altered material (Sharp and Cerling 1996; Cerling and Sharp 1996).

Laser Ablation

Laser ablation is a relatively new technique that allows *in situ* oxygen and carbon

isotopic analysis of phosphate and carbonate (Sharp and Cerling 1996). That is, the sample does not need to be crushed and reprecipitated and small areas on the material may be analyzed as is. This allows for finer spot analysis than had been possible previously with traditional methods that required the homogenization of large amounts of material. The major advantages of this technique are that it is essentially non-destructive and requires little sample preparation. However, it may be used for analysis of the inorganic hydroxyapatite alone, and not collagen due to the nature of the process.

There are different types of lasers that may be used. One such laser ablation method uses infrared (IR) heating and produces carbon dioxide gas (CO_2). Rapid heating to high temperatures occurs using a focused laser beam. CO_2 is generated by the decomposition of the sample due to the high temperatures: carbon released from the carbonate and oxygen released from both the carbonate and phosphate groups in the hydroxyapatite (Cerling and Sharp 1996). This CO_2 gas is picked up by a carrier helium gas that continually passes over the sample. The helium containing the sample CO_2 then passes through a gas chromatograph where other potentially contaminating gases are separated from the sample gas on the basis of electrical charge (Sharp and Cerling 1996). The uncontaminated sample gas is then introduced into the mass spectrometer where the isotope ratios are determined.

The spot sizes that may be obtained with this laser are quite small and range from about 100 µm to 200 µm (Cerling and Sharp 1996). It's evident that this would enable the analysis of particular areas on a piece of bone of a tooth. However, one drawback is that the heat from the laser does create 'damage' halos that may be as large as 800 µm around the ablation pit (Sharp and Cerling 1996).

Another similar method of laser ablation uses a ultra-violet (UV) laser. This method is slightly different from the CO_2 system and in fact, may produce smaller ablation pits (~ 30 µm) in comparison to the relatively large pits of IR laser ablation (~ 100 µm). Rather than using an IR laser, this form of laser ablation uses a UV laser in F_2 gas to heat the sample. Similar to IR laser ablation, this method of sampling may be combined with gas chromatography for separation of contaminating gases and uses mass spectrometry to measure the isotope ratios (Jones *et al.* 1999). UV lasers do not result in damage halos around the ablated areas as may be present when using IR laser ablation (Cerling and Sharp 1996). The oxygen values obtained using these alternative methods have been shown to correlate with traditional Ag_3PO_4 values (Jones *et al.* 1999). However, it is obvious that even with these minor issues, the amount of sample preserved in the process and the resolution is much greater than traditional methods and this is the most valuable aspect of laser ablation for archaeological applications.

As indicated, there are a number of advantages associated with the use of laser ablation over other conventional methods although it is not yet in common use and there are few examples of actual applications. First, there is a reduction in the amount of sample preparation required versus other methods. There is no need to crush and reprecipitate samples with silver phosphate or to use a dangerous reactant such as BrF_5 (Cerling and Sharp 1996). There is a reduction in the amount of fractionation that occurs between CO_2 gas and the oxide as compared to conventional vacuum heating systems. This is due to the rapid high temperature heating of the sample by the laser (Cerling and Sharp 1996). Second, minimal damage to the samples means that very small samples and/or rare specimens may be analyzed without destruction of the entire sample. In fact, with small ablation areas such as those attainable with the UV laser, areas may be ablated multiple times without loss of precision (Jones *et al.* 1999). This is particularly beneficial to archaeological studies in which bone recovery is rare and any small amount of material is prized. And finally, the high resolution allows for variability in isotopic composition within a single sample such as a tooth to be studied which is an important aspect for use in archaeological analysis. These analyses allow for study of migration patterns, seasonality, changes in diet, climate, or physiology during the period of tooth growth. Teeth are beneficial in isotope studies as the oxygen isotope signatures are incorporated into the enamel at the time of mineralization during childhood and do not change over time providing researchers with information regarding where that individual was likely born and/or spent their childhood (White and Spence 1998). It is also known that the phosphate $\delta^{18}O$ and $\delta^{13}C$ of enamel will change during growth and initial mineralization with seasonality of formation, value of ingested water and the behavioural characteristics of the species (Bryant *et al.* 1995; Fricke and O'Neil 1996; Balasse *et al.* 1999). Analysis of layers within the teeth would provide

information about changing environmental conditions during the period of formation.

Evidence for change in diet has been found in intra-tooth variability of hydroxyapatite carbon isotopic composition. One study tested intra-tooth variability by measuring the carbon isotope values in modern bulls raised on restricted diets (Balasse *et al.*1999). However, this was not done using laser ablation and relatively large amounts of enamel were required for the analysis (approximately 40 – 50 mg of sample). The enamel hydroxyapatite samples were removed from the upper and lower portion of each tooth using a drill (Balasse *et al.* 1999). Changes in the controlled diet were reflected in the $\delta^{13}C$ values of the enamel. Although this study was able to demonstrate a distinct difference in the values before and after changes were made in the diet, it does not necessarily give a clear picture of exactly when and how quickly these changes occurred. In this case, it is evident that the use of laser ablation may have provided additional information. With laser ablation, smaller samples could have been taken along the tooth a regular intervals to produce a dietary profile rather than only taking samples from the ambiguous 'upper' and 'lower' portion. Such fine spatial sampling is not possible when using methods that require large sample sizes and are destructive in nature.

Phosphate oxygen variation within a single tooth has been reported previously by Fricke and O'Neil (1996). In this particular study, bison and sheep teeth were analyzed and enamel samples of approximately 40 mg were taken at ~ 3 mm intervals along each tooth by drilling. It is noted by the authors that the number of trials that could be run was limited by the size of the tooth. Comparatively, laser ablation requires sample sizes of only about 1 – 2 mg and, as indicated previously, produces ablation pits of 100 μm in diameter, use of this technique would have allowed a greater number of analyses (Jones *et al.* 1999). In another similar study using fossil animal teeth, CO_2 laser ablation has also been shown to identify variation in oxygen values from a single tooth by up to several per mil as reported similarly by earlier researchers using more labour intensive methods and requiring larger sample sizes (Cerling and Sharp 1996). This demonstrates that there is isotopic variation within teeth that may only be analyzed using a technique with fine spatial resolution such as laser ablation. This study also analyzed the oxygen isotope values in a number of the teeth using a traditional BrF_5 with Ag_3PO_4 method. These results were compared to those obtained using the CO_2 laser ablation and were found to correlate with a relatively high degree of precision (Cerling and Sharp 1996). Any small differences are potentially explained by natural heterogeneity within a single tooth, as has been observed by others (Fricke and O'Neil 1996).

In another study involving dentition, fossil horse teeth were analyzed using CO_2 laser ablation to gain seasonality information. Conventional ^{13}C and ^{18}O analyses of samples from the same area vary by as much as 4 ‰. The ability to analyze carbon and oxygen values over the course of a single year of tooth growth allowed the researchers to determine with high precision that the proportion of C_3 and C_4 plants varied during summer versus winter months (Sharp and Cerling 1998). As well, the measurement of variation within teeth over the course of development may allow for determination of long-term seasonal variation at a given site.

Other studies on dentition have been done using microdrills to sequentially sample teeth. However, with this manner of sampling, temporal resolution is a concern. The question has been raised as to whether or not this relatively bulk sampling procedure allows for the isolation of discrete periods of time as would be possible with laser ablation (Balasse 2003).

In addition to allowing for more detailed temporal profiling, the high spatial resolution of laser ablation may allow for the isotopic analysis of material that has been altered by diagenesis (post-depositional chemical alteration). The validity of isotope values of material suspected to have undergone diagenesis has long been debated (Zazzo *et al.* 2004). It is known that isotopic exchange with surrounding soil groundwater and other elements may occur in skeletal tissue, leading to a shift in the isotopic composition of the hydroxyapatite (Lee-Thorp 2002). The implications of this are that the isotopic composition obtained during analysis may not be a reflection of the true pre-burial signature (Ambrose 1993; Bell *et al.* 2001; Schoeninger *et al.* 2003).

Previous to technological advances such as laser ablation, such material was discarded from further analysis due to the possibility that the isotope values were not a true reflection of pre-burial conditions (Ambrose 1993). In some cases, the extent of alteration may be so great as to completely misrepresent an animal's feeding strategy (Schoeninger *et al.* 2003). However,

with the advent of laser ablation, it may be possible to analyze this material using small areas within the bone or tooth samples that have been identified as being non-altered. Although it has been shown that chemical alteration of bone may occur in as few as 5 days after burial, previous studies have also identified areas within bone samples that have survived apparently unaltered and not been affected diagenetically (Jackes *et al.* 2001; Zazzo *et al.* 2004). For example, one study found that bone from the Mesolithic period, although extensively altered by bacterial invasion, did have small areas that did not show evidence of alteration (Jackes *et al.* 2001). It is obvious that if this was the only human tissue found at this site, it would be quite valuable from a research perspective. However, the need for such large sample sizes with traditional methods of isotopic analysis and the need to crush and homogenize the sample would prevent an accurate analysis of unaltered material. Only the high spatial resolution available with laser ablation would allow for use of this material. Using traditional methods of analysis, it would be quite difficult to separate out these regions of potential isotopic value.

The stable isotope analysis of archaeological material is a fundamental tool in the reconstruction of past diets and study of migration patterns. Current methods of analysis using traditional tools provide a great deal of information to researchers, yet advances in technology allow for even more information to be gleaned from samples. It is evident that the isotopic analysis of archaeological material benefits greatly from the development of laser ablation as a means to sample bones and teeth. This relatively new method allows for the analysis of rare or small samples, finer spatial resolution, and potential use of diagenetically altered material. As such, it has opened new avenues for research into the stable isotope composition of archaeological material and allowed for greater applications towards palaeodiet and palaeoclimate reconstructions.

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